Infectious Processes in Plants

Development of Vesicular-Arbuscular Mycorrhizae

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I regard infection processes in vesicular-arbuscular (VA) mycorrhizae to include arrival of the fungus at the root, penetration and development of the infection, and then its spread to other parts of the root. Resistance to infection is the exception rather than the rule—not only are most families of higher plants susceptible but also several pteridophytes and bryophytes are infected (19). However, variation occurs between fungus–host combinations in the development of the infection. Compared with plant pathogens and legume nodules, research on VA mycorrhizae is recent, having developed mainly over the past decade. Despite some excellent electron microscope studies, the physiological bases of infection are not well understood. In this paper I comment on some of the approaches to analyzing the biology of the infection and on some of the physiological considerations.

The three cardinal phases of infection are indicated in Fig. 1a, from Furlan and Fortin (18), in which temperature is shown to affect all three phases: a lag phase (I), a period of rapid development of the infection (II), and a "plateau" phase (III) indicating the maximum percentage of root infected for that plant species (47). Figure 1b, from Abbott and Robson (1), shows that with some fungus–plant combinations infection is so rapid that phase I hardly exists.

PREINFECTION

The preinfection phase involves germination of spores (or other propagules), growth to the root, and possibly growth in the rhizosphere. However, growth in the rhizosphere is not demonstrably necessary for infection, although it will probably be involved in secondary infection along the root.

Soil factors such as pH, temperature, and nutrient level affect the amount of root infected with VA endophytes (18, 21, 25, 35, 44), but usually the effects on the individual steps—preinfection, the entry of the fungus, and spread—have not been defined. Overall environmental effects on the first two of these, which largely constitute phase I, can be initially examined together by using an experimental approach (e.g., 44) or by the mathematical analysis of infection (45). Experimentally it is important that numbers of infection points, or "infection units" (i.e., infections arising from the one entry point), be counted rather than the percentage of the root which is mycorrhizal—the usual measurement. The number of infection units on a root does not increase proportionally beyond a certain number of propagules (11) (which must be determined for each fungus–plant combination), and thus it is important that the inoculum be standardized to be somewhat below that point so that maximum sensitivity is obtained in experiments on environmental effects. Soil temperature strongly affects phase I (Fig. 1a; 44), as does soil moisture; Reid and Bowen (39) showed that a reduction of soil moisture from −0.19 MPa (22% moisture) to −0.43 MPa (15% moisture) reduced the number of infection points in *Medicago truncatula* by 77%. In clover, 500 mg of superphosphate per 3 kg of soil reduced numbers of infection points by 81 to 94% (25). Soil pH also affects phase I; e.g., it markedly effects germination of spores of VA fungi in soil (15).

The fungus species and the type of propagule can affect phase I greatly. Considerable variation occurs in the time for germination of spores; e.g., spores of *Acaulospora laevis* may take 1 to 2 months to germinate (38) whereas inocula consisting of infected roots can infect rapidly.

Experimental approaches to examining the individual factors in the preinfection phase (spore germination, germ tube growth, rhizosphere growth) are simple to devise and have been discussed earlier (8). Infection can be blocked at any one of these stages. I therefore stress only that studies in laboratory media can be quite misleading if extrapolated to soil.

The plant's role in stimulation of VA endophytes in soil is still unclear. Spores germinate in moistened soil in the absence of plant roots, but there is no comprehensive study of whether roots can enhance this. However, with spores on agar-covered glass slides in soil, directed growth of hyphae to onion root (presumably along a chemical gradient) occurred for some 3 to 4 mm with one fungus species and for 1.6 mm from spores of two other species (38). Koske (29) found attraction of germ tubes of *Gigaspora*

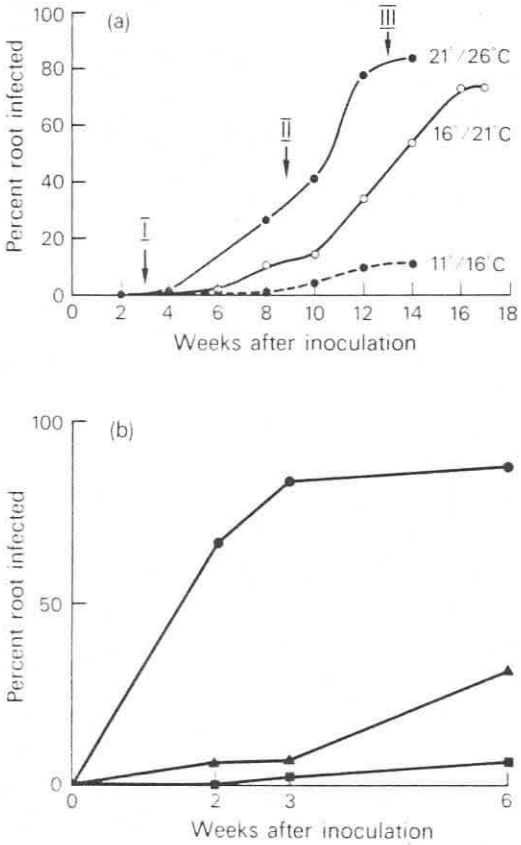

FIG. 1. Development of VA mycorrhizal infections. (a) Effect of 3 day/night temperatures (indicated) on infection of *Allium cepa* by *Gigaspora calospora*. Infection phases I, II, and III are indicated for 21°C/26°C. From Furlan and Fortin (18). (b) Development of infection by three endophytes on *Trifolium subterraneum*: ●, *Glomus fasciculatus*; ▲, fine endophyte; ■, *Acaulospora* sp. From Abbott and Robson (1).

gigantea to roots of bean and of corn by volatile agents from the roots.

INFECTION PROCESS

There are few studies of the infection process at the light microscope level, and there are almost no studies of the initial penetration of the root. Infection is often (not always) preceded by appressorium formation. Hyphae then penetrate the outer cells (sometimes through a root hair) or (commonly) pass between epidermal cells and become intracellular on reaching the second layer of cortical cells. Structural changes occur in the hyphae inside the root, where growth can be intercellular or intracellular.

Detailed reviews of the ultrastructure of VA mycorrhizae have been given by Carling and Brown (12) and by Scannerini and Bonfante-Fasolo (42). Most, if not all, studies have been on a few species of *Glomus*, only one of four or five genera producing VA mycorrhizae. Most of the ultrastructural studies are on the penetration of cortical cells and the subsequent formation of the arbuscule, the highly branched organ involved in the exchange of nutrients and metabolites with the plant. The other major structural body, the vesicle, is a one- to several-walled multinucleate ovoid body, usually some 50 by 70 μm, in which lipid storage is prominent and in which glycogen sometimes occurs. Vesicles may develop both within and between plant cells and appear to be incipient chlamydospores.

In arbuscule development, penetration starts as a constricted hyphal peg causing the cortical cell wall to stretch and invaginate (24). After penetration, the hypha resumes its normal size and dichotomizes frequently to produce the arbuscule. This is multinucleate, is always enclosed in the fungal cell wall (sometimes very thin), and has a relatively short life span, usually from 4 to 15 days (5, 14). A stretching of the host cell wall and alteration of the middle lamella during penetration (27, 28) suggest a fungus enzyme action in penetration of the cell wall (and possibly a plant reaction also) rather than a mechanical rupture of the cell wall. During arbuscule development the fungus plasmalemma invaginates and produces multivesiculate paramural bodies or lomasomes, which are particularly abundant in the arbuscule branch. These are rich in polysaccharides and have been suggested as important in exchanges of metabolites between fungus and plant (16). The arbuscule has many organelles and has inclusions of glycogen, lipid, and polyphosphate (12, 14, 28).

What of the plant's reaction to the infection? Many, but not all, of the reactions to biotrophic pathogens occur; e.g., papilla formation (3, 17) does not occur as a host response. The trunk of the arbuscule, however, is surrounded by a "collar," continuous with the plant cell wall and containing glycoproteins which are the same as those of the cell wall (16, 42). As the arbuscule develops, this collar becomes thinner and is replaced by a matrix of plant cell origin consisting largely of disorganized polysaccharide fibrils (16) that surrounds the entire arbuscule. The amount of this interfacial matrix diminishes with development of the arbuscule. Histochemical tests show that the matrix also contains some protein and possibly glycoprotein (6). Host cell wall deposition is not rapid enough to contain the fungus, which apparently disorients the ability of the plant to organize the fibrillar network into a cell wall. When growth of the arbuscule ceases, the fibrillar layer encases it.

The plant cell's reaction to the infection is slight; although phenolic-type deposition does

not occur (24), there are several detectable changes in the host cytoplasm. The highly invaginated host plasmalemma is elaborated into paramural bodies which can contain cytoplasm and which are cut off and occur near the fungus. Host cytoplasm is increased by up to 20-fold (14); the nucleus becomes highly polyploid and the nucleolus is greatly enlarged. Golgi activity increases markedly (no doubt involved in the secretion of the polysaccharide matrix), and the endoplasmic reticulum is also greatly increased. These changes reflect great metabolic activity of the host cell: VA mycorrhizae have been found to respire at a rate six times that of roots lacking the VA endophyte (F. E. Sanders, J. K. Martin, and G. D. Bowen, unpublished data). Plastid development in the cell is blocked at the protoplastid phase or plastids turn into chromoplasts, which suggests a modification of the carbohydrate metabolism of the root cell; Scannerini and Bonfante-Fasolo (42) pointed out that, although starch is usually absent from invaded cells, minute amyloplasts have been observed and the fungus may not prevent starch accumulation totally. In sharp contrast to plant pathogenic infections, no observable cytochemical or morphological difference in the host plasmalemma occurs after infection (6), a fundamental difference which may be crucial in the maintenance of a two-directional transfer of nutrients and metabolites between fungus and root.

PHYSIOLOGY OF INFECTION

In contrast to most plant pathogens, VA mycorrhiza fungi have an extremely wide range of hosts. With VA mycorrhizae the major phenomena to explain are the lack of specificity, the absence of typical infections in a few plant families, and the inhibition of infection by high soil P and by other environmental factors such as low light intensity.

Recognition and lack of specificity. The VA mycorrhiza symbiosis is very old indeed. Fossils of some of the earliest land plants indicate them to have had VA mycorrhizae (36). During evolution, this has conferred an effective selective advantage on mycorrhizal individuals in nutrient-poor sites. Infection is usually absent in wet conditions (21, 39), and if the dry land forms of families such as *Cyperaceae* and *Commelinaceae* (predominantly wet-site families) evolved separately from related plants such as the *Graminaeae*, this could explain the general absence of susceptibility to VA infection in *Cyperaceae* and *Commelinaceae*. These would then have evolved other mechanisms for coping with low-nutrient soils, e.g., production of many fine roots.

Nothing is really known about recognition phenomena in VA mycorrhizae and reasons for the lack of specificity. Physiological studies on this are difficult (but not impossible) because the fungi cannot yet be grown in vitro, although limited growth can be obtained from germinating spores (22). A first site of recognition may be at the cell wall, which could assist penetration by the stimulation of pectin-degrading (or other) enzymes in the fungus, causing the cell wall changes which occur during penetration. Enzyme activities of extra-matrical hyphae not involved in penetration, needed as a reference point, have not been performed. As with some plant pathogens, appressorium formation indicates a type of recognition, possibly caused by lectin-type reactions or polysaccharide–polysaccharide interactions; again, this has not been studied. However, appressorium formation could be only a thigmotrophic response, and in any case recognition does not necessarily incur appressorium formation; often appresoria do not occur with VA infection.

The most important site of recognition is probably between the fungus and the host plasmalemma, and this leads to secretion of the matrix and the increased host metabolic activities indicated above. As hypothesized by Sequeira (43) for *Agrobacterium*, a wide host range would be consistent with nonspecific recognition phenomena involving polymers common to many plant species. The stimulus for the host response is not known with VA mycorrhizae; e.g., it is not known whether recognition involves lectins of the host, lectins of the fungi, or polysaccharide–polysaccharide interactions. Polysaccharide and protein complexes of VA fungal cell walls can differ among closely related species (6), but obviously this does not result in differing specificity.

The fungus apparently prevents the host from assembling a cell wall from the matrix produced by the cell; more detailed study of this apparent inhibition may be rewarding. Also, VA mycorrhizal fungi should be examined for their ability to elicit phytoalexins; it is possible that they do not, for this would also necessitate either their indifference to a wide range of phytoalexins produced in higher plants or an ability to break down a wide range. The many and varied changes in the root cell with arbuscule formation are interesting mainly in their amount, rather than in their nature. This and the reversion of the host cell to "normality" after arbuscule degeneration suggest that the fungus may affect the production of growth regulators. The production of cytokinin and growth regulator compounds by VA mycorrhizal fungi is indirectly evidenced by the enlarged nucleolus of the plant cell and a possible effect on stomatal physiology (4). A hormone interaction with the plasmalemma, should it occur, would also enhance ex-

change of nutrients and metabolites via effects on permeability.

The apparent lack of infection of *Chenopodiaceae, Brassicaceae, Caryophyllaceae*, and a few other families could be due to any of numerous reasons, ranging from a physical barrier of the cell wall, to an absence of essential nutrients, to production of toxins by the plant. A lack of nutrients is not likely to be the major reason, for VA endophytes fungi can grow in the rhizosphere of nonhost plants (5, 15, 37). The occurrence of a wide range of S compounds in *Brassicaceae* and of betalins (related to phenols and anthocyanins) in *Chenopodiaceae*, with fungistatic activity, might be important. Infection of some species in these two families has been recorded (23, 30, 34, 37, 48), but in all but one case (48) infection was light; in no case did arbuscules form, and usually infection was in older roots and in the presence of a susceptible plant species, a most interesting observation.

The nonoccurrence of arbuscules in *Chenopodiaceae, Brassicaceae*, and some other plants, and the restriction of arbuscule formation to inner cortical cells in some plants (12, 24, 28), indicate some very specific cell recognition, fungus–host interactions within a root system. In what ways do some interior cortical cells differ physiologically and physically (e.g., oxygen level) from outer cortical cells? If single-gene mutants could be found which produce only vesicles in susceptible plant species, much might be learned about recognition and stimuli for arbuscules. If single-gene mutants of the cruciferous plant *Arabidopsis thaliana*, used so extensively in biochemical genetics research, were found which produce normal infections, they would be valuable material. Some infection of this plant has been recorded (30).

Effects of environment. Of the environmental factors which markedly affect formation of VA mycorrhizae, soil phosphate has received most study. Figure 2, from Jasper et al. (25), shows that increasing soil phosphate markedly decreases VA mycorrhizal infection and that VA mycorrhizal fungi differ in their sensitivity to increasing phosphate. The phosphate acts via the plant and not on the soil phases of the fungi (33).

Phosphate deficiency leads to an increase in the loss of sugars and amino acids from the root into the rhizosphere partly as a result of an increase in these compounds in deficient roots (7, 20) and partly as a result of increases in plasmalemma permeability (20) which sometimes, but not always, occurs (7). This greater "exudation" by low-phosphate plants has been correlated with a greater proportion of the root infected (some weeks later), and a causal relation with infection has been claimed with exu-

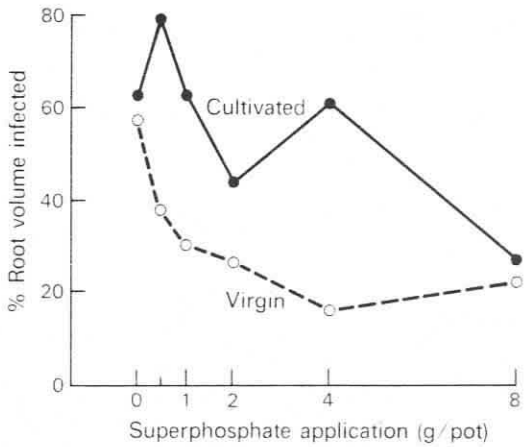

FIG. 2. Effect of soil phosphate on mycorrhizal infection of *Lolium rigidum* by the natural populations of: ●, a previously phosphate-fertilized, cultivated soil (endophyte: *Glomus monosporus*); ○, adjacent virgin soil (endophyte: *Acaulospora laevis*). From Jasper, Robson, and Abbott (25).

date composition rather than cell composition. The acceptance of this poses difficulties (20). In some instances there has been a correlation between percentage of the root which is mycorrhizal and total sugars in exudates and in other cases with amino acids (20, 26). Also, analysis of the whole root is a poor indicator of the chemical composition of the cytoplasm. "Exudates" probably reflect the composition of the cytoplasm, not the whole cell (with which correlations would be expected to be poorer). Losses of organic acids from the root often exceed those of sugars and amino acids (46), and these may warrant study. Furthermore, substances normally recorded as "exudates" come from several parts of the root (32). Although root exudation almost certainly assists spread of the fungus in the rhizosphere, there is as yet no good evidence that rhizosphere growth is necessary for infection. Neither does the plasmalemma–permeability–exudate theory comfortably accommodate a finding (40) that small amounts of phosphate increase the percentage of the root infected. If exudates are involved in the infection process, it is possible that it will not be by such common groups of compounds as those above but by specific substances (? elicitors of enzyme activity).

Jasper et al. have suggested (25) that phosphorus nutrition acts via effects on concentration of soluble carbohydrates within the cell. A good inverse relation was found between soluble carbohydrates of clover roots at various phosphate levels and the number of infection units 21 days later, but the relationship was less convincing for infection numbers at 14 days. In later studies

(40) a relationship was observed between soluble carbohydrates in roots (manipulated by temperature, light, and plant defoliation) and numbers of infections 6 days later. However, the strongest correlations were between carbohydrate in the whole root and the percentages of root infected 12 days later.

The best correlations obtained by protagonists of either of the two theories above are with the percentage of the root infected, which consists of infection and growth and spread in the root after infection. I am attracted to the "carbohydrate" theory as being important in growth in the root and spread of the infection. However, we are dealing with correlations (moreover, at a coarse level), and phosphate deficiency can affect many components of the cell. It may be interesting, for example, to examine effects of phosphate nutrition on glycerols and lipids, which may be important in the nutrition of *Endogonaceae* and related fungi (22, 31).

VA mycorrhizal fungi have defied all attempts to grow them. If one then hypothesizes that the plant provides not only energy sources but also (so far) unknown growth factors, it may well be that phosphate nutrition affects not only the level of soluble carbohydrates and other energy sources but also production of this factor(s)—whatever it might be.

SPREAD OF INFECTION

Plant response is often correlated with infection level of the root (2, 41), as this provides the energy sources for growth of the fungi into soil (and nutrient absorption). In field soil that contains VA fungus propagules, continued development of the infection occurs from spread of existing infections and from new infections. The slopes of phase II in Fig. 1a are a combination of these two phenomena. These must be separated in analyzing plant response to infection and its possible management. In inoculation programs, the fungus is added to a discrete part of the young root system (or the seed), and the spread of the infection, often in competition with indigenous VA mycorrhizal fungi, is important to a plant response. Competition between mycorrhizal fungi has been examined by Abbott and Robson (1) and by Wilson and Trinick (49) and will not be discussed further here.

Spread of the infection has received little study, although satisfactory methods for doing so experimentally are very simple. Some consider internal spread to be limited and refer to a study (13) with onion and one VA mycorrhiza fungus, in which a spread of less than 5 mm in each direction was recorded. However, other studies (5) have indicated that infection of onion by two fungus species was quite localized but that considerable spread occurred in clover.

Two complementary approaches to investigating spread have been developed. In the first (G. D. Bowen, unpublished data) plants are grown in soil in boxes with removable Plexiglas sides so that root segments of known ages can be inoculated locally and subsequent spread internally and externally can be examined. In the second approach, spread is derived from mathematical analyses (with certain assumptions) of the increase of infection of roots with time (9, 10, 45). Although the mathematical approach has considerable power, especially in distinguishing between spread and increased infection via new infections from soil (45), it cannot be stressed too strongly that hypotheses generated by such models must be verified by experimental approaches.

Smith and Walker (45) have mathematically dissected the spread of existing infection concomitant with new infections (from soil) in clover. The basic assumptions included that (within limits) any uninfected portion of the root is equally likely to become infected regardless of age and that all infections grow at the same rate regardless of age. These assumptions need direct experimental study. The number of infections, the total length of root, and the total length of root infected with time are used to derive the numbers of new infections per centimeter per day and the spread of the infection. The model indicated different effects of nitrogen and of chloride additions to soil on these two properties. The analyses of test data for the particular fungus–plant–soil combination suggested that secondary infections were of little consequence; this may well be so when high inoculum densities of fungi are used with which new infections occur rapidly, but if these conditions are not met, secondary infections may be very important and confound the analysis.

A second type of analysis by Buwalda et al. (9, 10) examined the spread of inoculum along roots from a point source. The assumption (and biological interpretation of the analyses) is made that this is mainly by growth in the rhizosphere leading to secondary infections (an assumption which needs testing). The equation used is formally identical to that of Smith and Walker (45). Measurements of total root length and infected root length at various times are used to calculate a spread rate S and a correction term n, accommodating the observation of several authors that different species have upper limits to the infection, phase III of Fig. 1a. The effects of phosphorus nutrition on these two parameters in wheat and in leek (*Allium porrum*), independent of the effect on root growth, were examined. Rate of spread in wheat was 37% faster than in leek and was not generally affected by phosphate level within species. The factor n was

affected markedly both by species and by soil phosphorus level: at low P, almost all the leek root but only half of the wheat root became infected; at higher soil P three-quarters of the leek root but less than one-tenth of the wheat root was infected. One area of concern is that the two models discussed here, developed with different assumptions of the biology, have essentially the same form. This reinforces the need to examine the biology of the system as well as the mathematics and to test generated hypotheses experimentally.

Little is known of the physiology of spread and of the determinants of maximum percentage of infected root. External spread in the rhizosphere will probably be affected both by translocation of substrates from the existing arbuscules and by the nature of the rhizosphere "exudates." The apparent lack of effect of soil P level on spread of the fungus in the studies of Buwalda et al. (10) (which was assumed to be external) is in conflict with data indicating greater "exudation" under low P conditions (20). It is highly likely, however, that spread both internally and externally will be affected by internal energy sources (e.g., soluble sugars) available in the root. The factor *n* may, as Buwalda et al. (10) indicated, be of major importance in the physiology of the symbiosis. Differences in *n* could indicate that root segments of some species are susceptible for only a short time. However, they might also reflect the cardinal importance of "balanced development," which is important in a mutualistic symbiosis. Factors such as the distribution of plant assimilate between host cell and fungus possibly determine both *n* and the longevity of the arbuscule; this, I believe, deserves more detailed physiological study.

CONCLUSIONS

To understand the factors involved in infection processes in VA mycorrhizae, it will be necessary to use experimental approaches to analyze the three separate phases: preinfection growth to the root, initiation of the infection, and subsequent spread (and growth of the fungus into soil). The construction of curves relating soil populations of the fungi to the number of infections is basic to defining factors affecting the first two of these. Mathematical analysis of the increase of infection with time is a powerful emerging tool in distinguishing spread of existing infections from increases due to new infections and in indicating the effects of soil and plant factors on these. However, the assumptions used to develop the analyses, and the hypotheses they generate, must be tested experimentally and not embraced uncritically. Such methods being developed for "epidemiology" analysis of VA mycorrhizal infections will also be directly applicable to the analysis of root pathogenic infections.

The great lack of specificity and the mildness (and subtlety) of the plant cell reaction to infection are outstanding physiological phenomena in VA mycorrhizal infections. Relatively little is known about the biochemistry of penetration and "recognition" phenomena. An inhibition of host cell wall assembly appears confirmed. There are indications that the fungus increases polysaccharide production by the host and also increases host cell metabolism, but there is little knowledge of the mechanism of this or of whether the host cell produces reaction substances such as phytoalexins. There is also little knowledge of possible growth factors produced by plant cells which enable their growth of the fungus, in sharp contrast to our inability to grow the fungi in the absence of a plant.

Much of the data from physiology studies relating infection to environmental factors, such as plant phosphate status, relate to development and spread of infection rather than the infection processes themselves. The spread of infection in the root and the longevity of arbuscules are basic to abundant growth of hyphae from VA mycorrhizae into soil (and nutrient uptake for the plant). Understanding the physiological control of these, probably in relation to photosynthate distribution along the root, is an important area for future research.

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